Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

1. (currently amended) A method of isolating adult cardiac cells comprising,

(a) obtaining a tissue sample from a subject,

(b) successively exposing the tissue to a first solution with ~~decreasing~~ amounts of $CaCl_2$ decreasing from about 1-2 μM, comprising NaCl, HEPES, $MgCl_2$, KCl, and sugar at a pH of approximately 7.4,

(c) disassociating the tissue with an enzyme solution,

(d) repeatedly resuspending the disassociated tissue into a second solution with ~~increasing~~ amounts of $CaCl_2$ increasing from about 1-2 μM, comprising modified Earle's ~~modified salt~~, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, ~~fetal bovine serum,~~and an antibiotic, ~~and a fatty acid,~~ at a pH of approximately 7.4 to obtain isolated cells.

2. (currently amended) The method of claim 1, further comprising the step of re-suspending the isolated cells approximately every 24 hours in a solution comprising modified Earle's ~~modified salt~~, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, ~~fetal bovine serum,~~ an antibiotic, ~~a fatty acid acid,~~ and $CaCl_2$ at a pH of approximately 7.4.

3. (original) The method of claim 1, further comprising the step of incubating the isolated cells in a mixture of carbon dioxide and air.

4. (original) The method of claim 3, wherein the isolated cells are incubated at approximately 37°C.

5. (original) The method of claim 1 wherein, the first solution is exposed to the tissue at approximately 37°C and at approximately 4 ml/min for 3 minutes.

6. (original) The method of claim 1 wherein the concentration of $CaCl_2$ in the first solution decreases.

7. (original) The method of claim 1 wherein the first solution comprises approximately 140 mM NaCl, approximately 10 mM HEPES, approximately 1 mM $MgCl_2$, approximately 5.4 mM KCl, and approximately 10 mM D-glucose.

8. (original) The method of claim 1 wherein the enzyme solution comprises a digestive enzyme.

9. (original) The method of claim 8, wherein the digestive enzyme is a protease or a collagenase.

10. (original) The method of claim 1 wherein the concentration of $CaCl_2$ in the second solution increases.

11. (original) The method of claim 1 wherein the enzyme solution comprises approximately 140 mM NaCl, approximately 10 mM HEPES, approximately 1 mM $MgCl_2$, approximately 5.4 mM KCl, and approximately 10 mM D-glucose.

12. (currently amended) The method of claim 1 wherein the second solution comprises modified Earle's ~~modified salt~~, L-glutamine, sodium bicarbonate at approximately 1250mg/l, sodium pentothenate, creatine at approximately 328 mg/500ml, taurine at approximately 312mg/500ml, Ascorbic acid at approximately 8.8 mg, HEPES at approximately 2.383g/500ml, ~~fetal bovine serum at approximately 10% v/v,~~ and an antibiotic at approximately 5% v/v, ~~a fatty acid at approximately 1 μM~~ at a pH of approximately 7.4.

13. (currently amended) A method of isolating adult cardiac cells comprising,

(a) obtaining a tissue sample from a subject,

(b) successively exposing at approximately 37°C the tissue to a first solution with ~~decreasing~~ amounts of $CaCl_2$ decreasing from about 1-2μM, comprising approximately 140 mM NaCl, approximately 10 mM HEPES, approximately 1 mM $MgCl_2$, approximately 5.4 mM KCl, and approximately 10 mM sugar at a pH of approximately 7.4,

(c) disassociating the tissue with an enzyme solution for approximately 8 minutes comprising approximately 140 mM NaCl, approximately 10 mM HEPES, approximately 1 mM

$MgCl_2$, approximately 5.4 mM KCl, and approximately 10 mM sugar, to form disassociated cells,

(d) repeatedly resuspending the disassociated cells into a second solution with ~~increasing~~ amounts of $CaCl_2$ increasing to about 1-2μM, comprising modified Earle's ~~modified salt~~, L-glutamine, sodium bicarbonate at approximately 1250mg/l, sodium pentothenate, creatine at approximately 328 mg/500ml, taurine at approximately 312mg/500ml, ascorbic acid at approximately 8.8 mg, HEPES at approximately 2.383g/500ml, ~~fetal bovine serum at approximately 10% v/v,~~ and an antibiotic at approximately 5% v/v, ~~and a fatty acid at approximately 1 μM~~ at a pH of approximately 7.4 to form a solution of isolated cells,

(e) incubating the isolated cells in a mixture of carbon dioxide and air at approximately 37°C, and

(f) re-suspending the isolated cells approximately every 24 hours in a solution comprising modified Earle's ~~modified salt~~, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, ~~fetal bovine serum,~~ an antibiotic, ~~a fatty acid,~~ and $CaCl_2$ at a pH of approximately 7.4 to obtain isolated cells.

14. (withdrawn) A method of cultivating isolated cells comprising, resuspending the isolated cells approximately every 24 hours in a solution comprising Earle's modified salt, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, fetal bovine serum, an antibiotic, a fatty acid, and $CaCl_2$ at a pH of approximately 7.4.

15. (withdrawn) The method of claim 14 wherein the solution comprises sodium bicarbonate at approximately 1250mg/l, creatine at approximately 328 mg/500ml, taurine at approximately 312 mg/500ml, ascorbic acid at approximately 8.8 mg/500 ml, HEPES at approximately 2.383 g/500ml, fetal bovine serum at approximately 10% v/v, an antibiotic at approximately 5% v/v, and a fatty acid at approximately 1 μM, and approximately 1mM $CaCl_2$.

16. (withdrawn) A cell culture media for cells comprising Earle's modified salt, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, fetal bovine serum, an antibiotic, a fatty acid, and $CaCl_2$ at a pH of approximately 7.4.

17. (withdrawn) The cell culture media of claim 16 wherein the media comprises sodium bicarbonate at approximately 1250mg/l, creatine at approximately 328 mg/500ml, taurine at approximately 312 mg/500ml, ascorbic acid at approximately 8.8 mg/500 ml, HEPES at

approximately 2.383 g/500ml, fetal bovine serum at approximately 10% v/v, an antibiotic at approximately 5% v/v, a fatty acid at approximately 1 μM, and approximately 1mM $CaCl_2$.

18. (withdrawn) A method of isolating cells comprising,

(a) obtaining a tissue sample comprising cells from a subject ;

(b) chopping the tissue;

(c) incubating the tissue in a first solution comprising calcium, salts, magnesium sulfate, pyruvate, glucose, taurine, HEPES, and nitrilotriacetic acid;

(d) incubating the tissue in a second solution comprising calcium, salts, magnesium sulfate, pyruvate, glucose, taurine, HEPES, and a digestive enzyme;

(e) incubating the tissue in a third solution comprising calcium, salts, magnesium sulfate, pyruvate, glucose, taurine, HEPES, and a digestive enzyme; and

(f) centrifuging the tissue to obtain isolated cells .

19. (withdrawn) The method of claim 18, further comprising the step of resuspending the isolated cells in a culture media comprising medium M199, BSA, ascorbic acid, taurine, carnitine, creatinine, insulin, and an antibiotic .

20. (withdrawn) The method of claim 19, wherein the culture media further comprises a fatty acid or magnesium.

21. (withdrawn) The method of claim 18, wherein the first solution comprises approximately 1-2 μM $CaCl_2$, approximately 120mM NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM $MgSO_4$, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and approximately 5 mM nitrilotriacetic acid, at a pH of approximately 6.96.

22. (withdrawn) The method of claim 18, wherein the second solution comprises approximately 1-2 μM $CaCl_2$, approximately 30 μM NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM $MgSO_4$, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme.

23. (withdrawn) The method of claim 18, wherein the third solution comprises approximately 1-2 μM $CaCl_2$, approximately 30 μM NaCl, approximately 5.4 mM KCl 5.4,

approximately 5 mM $MgSO_4$, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme.

24. (withdrawn) A method of isolating cells comprising,

(a) obtaining a tissue sample comprising cells from a subject ;

(b) chopping the tissue;

(c) incubating the tissue in a first solution comprising approximately 1-2 μM $CaCl_2$, approximately 120mM NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM $MgSO_4$, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and approximately 5 mM nitrilotriacetic acid, at a pH of approximately 6.96;

(d) shaking the tissue at approximately 37°C for approximately 12 minutes;

(e) bubbling approximately 100% O_2 through the solution;

(f) incubating the tissue in a second solution comprising approximately 1-2 μM $CaCl_2$, approximately 30 μM NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM $MgSO_4$, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme;

(g) incubating the solution in a third solution comprising third solution comprises approximately 1-2 μM $CaCl_2$, approximately 30 μM NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM $MgSO_4$, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme; and

(h) centrifuging the tissue to obtain isolated cells.

25. (withdrawn) A method of isolating and cultivating human myocardial cells comprising,

(a) obtaining a tissue sample comprising myocardial cells from a human subject;

(b) chopping the tissue;

(c) incubating the tissue in a first solution comprising approximately 1-2 μM calcium, approximately 120mM NaCl, approximately 5.4 mM KCl, approximately 5 mM $MgSO_4$, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and approximately 5 mM nitrilotriacetic acid, at a pH of approximately 6.96;

(d) shaking the tissue at approximately 37°C for approximately 12 minutes;

(e) bubbling approximately 100% O_2 through the solution;

(f) incubating the tissue in a second solution comprising approximately 1-2 μM, approximately 30 μM NaCl, approximately 5.4 mM KCl, approximately 5 mM $MgSO_4$, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme;

(g) incubating the solution in a third solution comprising third solution comprises approximately 1-2 μM, approximately 30 μM NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM $MgSO_4$, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 400U/ml of a digestive enzyme;

(h) centrifuging the tissue to obtain isolated cells;

(i) repeatedly resuspending the disassociated cells into a second solution which comprises increasing amounts of $CaCl_2$, Earle's modified salt, L-glutamine, sodium bicarbonate at approximately 1250mg/l, sodium pentothenate, creatine at approximately 328 mg/500ml, taurine at approximately 312mg/500ml, ascorbic acid at approximately 8.8 mg, HEPES at approximately 2.383g/500ml, fetal bovine serum at approximately 10% v/v, an antibiotic at approximately 5% v/v, and a fatty acid at approximately 1 μM at a pH of approximately 7.4 to form a solution of isolated cells; and

(j) incubating the isolated cells in a mixture of carbon dioxide and air at approximately 37°C.

26. (withdrawn) A method of isolating and cultivating rodent myocardial cells comprising,

(a) removing the heart of a rodent;

(b) perfusing the heart with low calcium Tyrode's solution for approximately 3 minutes;

(c) perfusing the heart with an enzymatic solution for approximately 8 minutes;

(d) perfusing the heart with a low calcium solution for approximately 3 minutes;

(e) removing the ventricles;

(f) mincing the ventricles to isolate myocardial cells;

(g) mixing the cells in a low calcium solution;

(h) resuspending the cells in a solution comprising increasing concentrations of calcium; and

(i) resuspending the cells in culture media solution.